OBSERVATIONS ON THE VEGETATIVE GROWTH OF ACTINOMYCETES IN THE SOIL.

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(Two Text-figures.)

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Introduction.

The development of actinomycetes in soils to which different kinds of organic matter had been added, was shown in earlier experiments (Jensen, 1935, 1936) to be favoured by conditions of high temperature and low moisture content. This was generally true of the actual numbers of actinomycetes, which were highest at 28° to 37°C. and very low at 5°C., as well as their proportional numbers in comparison with the bacteria. Also in soil samples taken from the field, the numbers of actinomycetes increased with decreasing soil moisture (Jensen, 1934). These results were secured by the method of colony counting on agar media, which does not differentiate between conidia and fragments of vegetative mycelium. Consequently the observed increases in numbers of actinomycetes, absolute or relative, might be due to a stronger tendency of these organisms to produce conidia when exposed to high temperature and growing in a relatively dry medium. Unless the growth of vegetative mycelium were also shown to be stimulated, the figures therefore could not be taken as proof that the quantitative importance of the actinomycetes in the transformation of organic matter in the soil also increases under the said conditions. It may here be recalled that fungi, which represent a quite close analogy to the actinomycetes in respect of conidia and vegetative mycelium, were actually found to produce their most abundant vegetative growth in soil at 5° to 15°C., whereas much higher plate counts, due to rapid formation of conidia, were observed at 37°C. Little experimental work has so far been done in this direction. The formation of conidia by actinomycetes in pure cultures was found by von Plotho (1940) to be stimulated in a dry atmosphere, and Cholodny (1936) observed that low moisture content of soil favoured the vegetative growth of actinomycetes; no quantitative determinations were made.

Experimental.

In order to gain some additional insight into the relation between the extent of vegetative growth and the numbers of colonies counted on agar medium, the density of *Actinomyces* mycelia was determined on a number of microscopic slides which in earlier experiments (Jensen, 1935, 1936) had been used for determining the density of fungal mycelium in soil under different experimental conditions. The method was essentially the same as previously used for the fungal mycelia: a number of microscopic fields evenly distributed over the slide were surveyed, and the density of *Actinomyces* mycelium was expressed as the percentage of fields showing the presence of vegetative hyphae; structures which could obviously be recognized as aerial hyphae by their larger diameter, more even outline and frequent division into conidia, were not counted as positive. The same method has been used by Garrett (1938) and Wieringa (1939) for estimating the abundance of actinomycetes in soil. Preliminary examinations showed that the actinomycetes were mostly less abundant than the fungal hyphae. Consequently the quadrat was made somewhat larger (approximately 0·01 mm.^{2}) than for the fungi, in order to get a percentage of positive fields high enough to give a

reasonable accuracy without examining an unduly large number of fields; as a rule 250 to 300 fields were examined on the richer, and 400 to 450 fields on the poorer slides. Some duplicate slides showed good agreement, as seen from the following percentages and standard errors of mycelial density:

1.—*a.* 35·9 ± 2·37	3.—*a.* 8·7 ± 1·35	5.—*a.* 5·3 ± 1·29
b. 34·0 ± 2·73	*b.* 7·7 ± 1·35	*b.* 4·8 ± 1·35
2.—*a.* 32·1 ± 2·90	4.—*a.* 8·2 ± 1·31	6.—*a.* 4·4 ± 1·25
b. 30·7 ± 2·00	*b.* 5·7 ± 1·11	*b.* 4·0 ± 1·24

A series of figures was obtained from an experiment with a loamy soil of pH 5·5, plus 1% hay meal, incubated for 12 days at three temperatures and with three degrees of moisture, corresponding to roughly one-half, two-thirds and four-fifths of the water-holding capacity (Jensen, 1935, Table 1, X). The mycelial densities, with standard errors, are seen in Table 1, which shows that an increase from room temperature

TABLE 1.

Influence of Temperature and Moisture on Vegetative Growth of Actinomycetes in the Soil.

Temperature and Time of Incubation.		Moisture Content of Soil %.		
		18	25	30
16–19° C.	5 days ..	4·4±1·25	2·3±0·83	2·0±0·89
	12 „ ..	11·5±1·94	17·9±2·29	17·3±2·35
27° C.	5 days ..	—	46·4±3·15	16·8±2·36
	12 „ ..	57·2±3·13	25·9±2·67	10·4±1·89
39° C.	5 days ..	40·2±3·10	21·3±2·36	10·4±1·89
	12 „ ..	25·8±2·71	27·3±2·76	13·8±2·14

(16°–19°C.) to 27°C. is accompanied by a very strong increase in the vegetative growth of actinomycetes, especially at low moisture. At 39°C. the development of mycelium is again somewhat less than at 27°C., and at both of these temperatures there is a significant reduction of vegetative growth with increasing moisture. No such influence of the moisture—if anything, the reverse—is noticeable at 16°–19°C., where the temperature seems to be the limiting factor, and where the growth has increased markedly after 12 days. It is thus possible that the density of mycelium would after still longer incubation have equalled, or even exceeded, the values at higher temperatures. Another set of data was therefore obtained from soils which had been incubated for a somewhat longer time and over a wider range of temperature. Particulars about these experiments are given elsewhere (Jensen, 1936, Table 4). Slides from the following soils were examined:

1. Sand-mixed grey loam, pH 7·7, + 1% dry mycelium of *Penicillium*.
2. "Synthetic" soil (sand-kaolin-mixture) + 1% dry mycelium of *Penicillium*.
3. Same soil as (1), + 1% hay meal.
4. Red loam, pH 6·0, + 1% hay meal.

Each soil was tested only at one degree of moisture, roughly two-thirds of its water-holding capacity. The results are seen in Table 2.

The figures show that the vegetative mycelium of actinomycetes develops most abundantly at 28°C. and 37°C., except in Exp. 3, where the growth at 15°C. eventually reaches the same density as at the higher temperatures; at 5°C. there is very little or no growth, even after prolonged incubation (Exp. 2). So far the microscopic method thus confirms in a general way the results found by plate counting. Otherwise there is no definite correlation between mycelial density and plate counts; it is particularly noteworthy that the vegetative growth declines more or less rapidly, especially at the higher temperatures, while the plate counts often remain at a high level for a considerable time after reaching their maximum. Fig. 1 shows a typical example. This phenomenon may be partly due to increasing fragmentation of the hyphae, but probably more to progressive formation of conidia which were often seen in large numbers on the slides, even after the decline in mycelial growth. The rise and fall of this is influenced

TABLE 2.

Density of Actinomyces *Mycelium in Soils incubated at Different Temperatures.*

	Incubation Days.					
1. Grey loam + fungal mycelium incubated at:	3	7	11	16	22	28
5° C.	—	0·5	0·2	0·4	1·8	1·6
15° C.	2·0	5·1	9·8	17·1	9·2	3·5
28° C.	35·0	8·0	4·2	—	—	—
37° C.	31·4	—	—	—	—	—

	Incubation Days.					
2. "Synthetic" soil + fungal mycelium incubated at:	4	8	14	20	28	40
5° C.	—	—	(<0·3)	(<0·3)	(<0·3)	(<0·2)
15° C.	—	0·8	1·5	1·2	0·3	—
28° C.	3·9	5·8	0·9	—	—	—
37° C.	9·9	—	—	—	—	—

	Incubation Days.					
3. Grey loam + hay meal iucubated at:	3	7	11	16	22	29
15° C.	0·5	1·9	1·8	13·1	9·8	1·7
28° C.	7·0	6·7	4·2	3·1	2·3	1·1
37° C.	8·0	11·0	4·8	2·8	3·1	—

	Incubation Days.			
4. Red loam + hay meal incubated at:	3	7	11	17
15° C.	2·0	6·8	1·7	—
37° C.	9·5	4·6	0·7	2·9

by the temperature in an interesting manner, as shown in Fig. 2. At 28°C. the rapidly-occurring maximum of mycelial density is seen to coincide with the peak of CO_2 evolution which indicates that at this time the decomposition of organic matter is going on with maximal intensity. At 15°C., on the other hand, the maximal growth of actinomycetes is not reached until well after the peak period of decomposition as indicated by the CO_2 evolution; here the actinomycetes thus seem to represent a kind of secondary decomposing flora which appears to attack the more resistant constituents of the added organic material or the cell substance of those organisms that have developed during the preceding stages of decomposition (cf. Ziemecka, 1935).

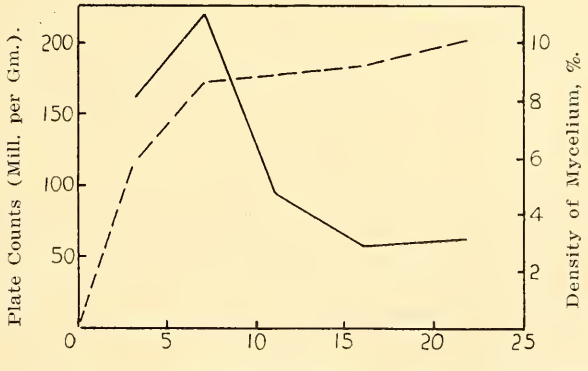

Fig. 1.—Comparison between density of vegetative mycelium and plate counts of actinomycetes in grey loam + hay meal (Exp. 3, Table 2) at 37°C. Continuous line: density of mycelium. Broken line: plate counts.

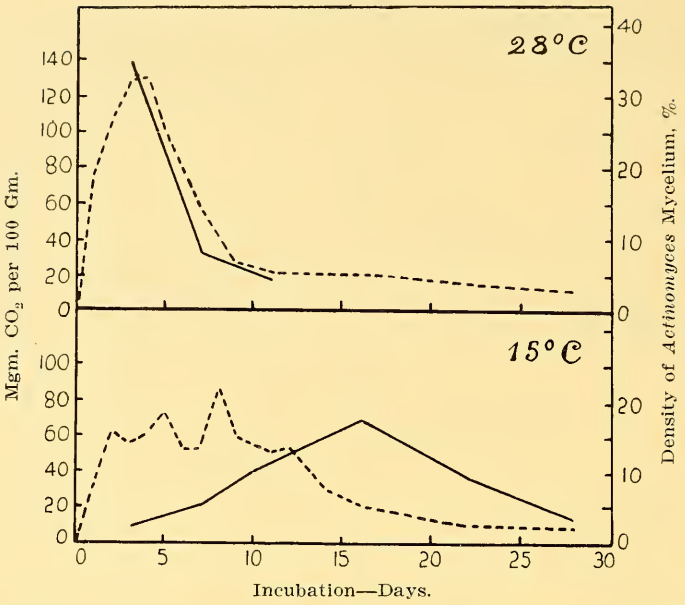

Fig. 2.—Mycelial growth of actinomycetes and carbon dioxide production in grey loam + fungal substance at 15° and 28°C. Continuous line: density of mycelium. Broken line: evolution of carbon dioxide, mgm. per 100 gm. dry soil in 24 hours.

The direct microscopical observations thus show that the actinomycetes as a group of the soil population develop most abundantly at 28° to 37°C., and are almost inactive at 5°C., although some species are able to grow at this and lower temperatures (Haines, 1932). In this respect they differ from the soil populations of bacteria and fungi which, if given sufficient time, reach their greatest density at 5° to 15°C. Also a relatively low moisture content of the soil favours the vegetative growth as well as the conidia formation. Thus there can hardly be any doubt that that part of the total decomposition processes in the soil which is carried out by the actinomycetes, increases with increasing temperature and decreasing moisture. On the other hand, their density on the slides is, even at 28°C., mostly less than that of the fungi (cf. also Garrett, 1938, and Wieringa, 1939), and since their hyphae are only about 0·5–1·0μ thick, the total amount of *Actinomyces* protoplasma seems small in comparison with the mass of fungi, whose hyphae mostly have a diameter of 2–4μ. Unless the rate of metabolism per unit volume of protoplasm is far higher than in the fungi, it therefore appears that the actual part played by the actinomycetes in the breakdown of complex organic materials is rather small in comparison with that of the fungi. A different state of affairs might obtain when compounds particularly stimulative to actinomycetes are undergoing decomposition (Ziemecka, 1935), or when a thermophilic microflora is active (Waksman *et al.*, 1939).

Summary.

Microscopic examination by means of the contact-slide method showed that the development of vegetative mycelium of actinomycetes in soil is favoured by relatively low moisture content and by increase in temperature between 5° and 28°C. A temperature of 37°C. caused no significant further stimulation in the vegetative growth, which at 5°C. was very scant or altogether absent. The actinomycetes, as a broad group of the soil population, thus seem adapted to a somewhat higher range of temperature than the fungi and bacteria, which attain their greatest abundance at 5° to 15°C. This confirms the results found by means of plate counting; otherwise there was no general correlation between the plate counts and the density of vegetative mycelium.

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